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High-quality ChIP-seq analysis of MBD3 in human breast cancer cells

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ABSTRACT

Chromatin accessibility is tightly regulated by multiple factors/mechanisms to establish different cell type-specific gene expression programs from a single genome. Dysregulation of this process can lead to diseases including cancer. The Mi-2/nucleosome remodeling and deacetylase (NuRD) complex is thought to orchestrate chromatin structure using its intrinsic nucleosome remodeling and histone deacetylase activities. However, the detailed mechanisms by which the NuRD complex regulates chromatin structure *in vivo* are not yet known. To explore the regulatory mechanisms of the NuRD complex, we mapped genome-wide localization of MBD3, a structural component of NuRD, in a human breast cancer cell line (MDA-MB-231) using a modified ChIP-seq protocol. Our data showed high quality localization information (*i.e.*, high mapping efficiency and low PCR duplication rate) and excellent consistency between biological replicates. The data are deposited in the Gene Expression Omnibus (GSE76116).

Specifications	
Organism/cell line/tissue	*Human/MDA-MB-231(ATCC)/Breast*
Sex	*Female*
Sequencer or array type	*Illumina NextSeq 500*
Data format	*Raw fastq and processed bigwig files*
Experimental factors	*N/A*
Experimental features	*ChIP-seq analysis*
Consent	*N/A*
Sample source location	*N/A*

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=mvwzocmmtlshxcp&acc=GSE76116

2. Experimental design, materials and methods

2.1. Cell culture

Human breast cancer cells (MDA-MB-231) were obtained from the American Type Culture Collection (ATCC) and maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) at 37 °C in a 5% CO_2 atmosphere.

2.2. Cell fixation

Sub-confluent MDA-MB-231 cells (two 15 cm dishes, treated separately) were washed twice with PBS, trypsinized, collected by centrifugation and washed once again with PBS. Cells were resuspended in 10 mL of 1% formaldehyde-PBS and incubated with constant inversion for 10 min at room temperature. To terminate fixation, 2 mL of 2 M Glycine was added to the solution and incubated for another 5 min at room temperature. Cells were divided into 3 tubes, washed twice with ice-cold PBS and stored at −80 °C until use.

2.3. Chromatin immunoprecipitation

To perform high quality ChIP-seq, we introduced two major modifications to a standard ChIP/ChIP-seq protocol [1]. First, we omitted sodium dodecyl sulfate (SDS) from all procedures because SDS can compromise the antibody-target protein interaction. Second, we incorporated a micrococcal nuclease (MNase) digestion step prior to chromatin fragmentation to maximize protein extraction efficiency. Detailed procedures are described below.

Fixed cells were thawed on ice and resuspended in 1 mL of ice-cold TM2 (10 mM Tris, pH 7.4, 2 mM $MgCl_2$) supplemented with 0.6% NP-40 and protease inhibitor [2]. After 10 min incubation on ice, cells were washed once with ice-cold TM2 and resuspended in MNase digestion

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Table 1
Basic quality score for ChIP-seq data.

Sample	Sequencing type	Raw read pairs (a)	Uniquely mapped pairs (b)	(b/a) %	Non-duplicate pairs (c)	(c/b) %
Replicate 1	Paired-end	50,887,944	35,909,398	70.57%	29,442,026	81.99%
Replicate 2	Paired-end	74,312,781	51,458,955	69.25%	44,711,162	86.89%

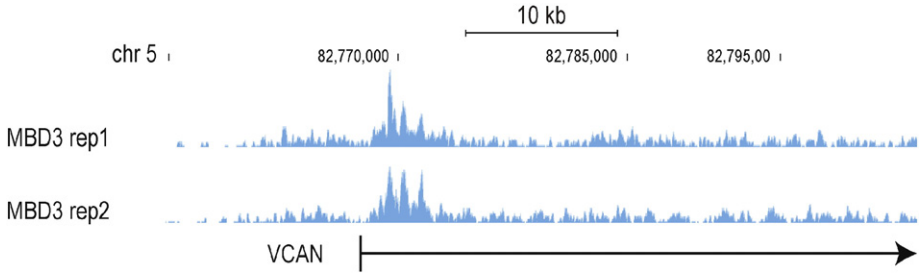

Fig. 1. An example in UCSC genome browser format. MBD3 binding pattern at VCAN gene is shown (two biological replicates; MBD3 rep1 and MBD3 rep2).

buffer (TM2 supplemented with 2 mM $CaCl_2$, protease inhibitor, and 4 U MNase). After thorough mixing, cells were digested at 37 °C for 3 min and then immediately placed on ice. To stop MNase digestion, 20 μL of 0.5 M EDTA was added. After 10 min incubation on ice, cells were centrifuged and resuspended in 1 mL of fragmentation buffer (TM2 supplemented with 600 mM NaCl, 1% Triton-100, 0.1% sodium deoxycholate, and protease inhibitor) and incubated on ice for 20 min. Then, cells were fragmented using focused-ultrasonication (Covaris) under the following conditions: 12 × 12 mm round-bottom glass tube (cap with AFA fiber), Duty Cycle 20%, Peak incident Power 145 Watts, cycles per burst 200, time 15 min, Bath Temperature 4 °C. The unsoluble fraction was removed by centrifugation and supernatant was collected as the chromatin fraction. The NaCl concentration was adjusted to 150 mM by adding TM2 supplemented with protease inhibitor. After 5 min incubation on ice, any unsoluble proteins were removed by centrifugation. Chromatin (600 μL) was incubated with 3 μg of anti-MBD3 antibody (Abcam, ab91458) at 4 °C for 16 h. After adding 15 μL of blocked Dynabeads protein A/G, chromatin fraction was further incubated at 4 °C for 1.5 h. Beads were washed three times with SDS-free RIPA buffer (25 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate), three times with SDS-free high salt RIPA buffer (25 mM Tris–HCl, pH 7.4, 600 mM NaCl, 1% NP-40, 1% sodium deoxycholate), and once with EB (10 mM Tris, pH 7.4).

2.4. Library preparation and sequencing

Washed beads were resuspended with Transposase buffer (20 μL of 1 × tagmentation buffer, 10 μL of Nextera XT transposase, 10 μL of EB) and incubated at 37 °C for 30 min. Beads were washed twice with SDS-free RIPA buffer, resuspended in 200 μL of SDS-free RIPA buffer, and incubated at 65 °C for 45 min. Samples were then treated with proteinase K, and the DNA was purified using DNA Clean & Concentrato-5 (Zymo Research) and eluted in 28 μL resuspension buffer. DNA was amplified and purified using standard NexteraXT PCR conditions without any modifications. The resulting libraries were sequenced on the Illumina NextSeq 500 paired-end system at the NIEHS Epigenomics Core Facility.

2.5. Sequencing data processing for quality control

Sequencing reads were quality filtered, adapter trimmed, and mapped to human reference genome (UCSC assembly hg19, GRCh37) using Bowtie (version 0.12.8) [3]. The non-duplicate uniquely mapped reads were selected with the Picard tool (http://picard.sourceforge.net) and used for subsequent analysis. Basic quality control data are provided in Table 1. To make coverage tracks, the paired-end reads were merged into single fragments and converted to bigwig files. An example locus showing MBD3 enrichment is depicted in genome browser format (Fig. 1).

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